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Laxative effect of peanut sprout extract

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Abstract

Certain phenolic compounds are known to exhibit laxative properties. Seed sprouts, such as those of peanut, are known to promote *de novo* biosynthesis of phenolic compounds. This study was conducted to examine the potential laxative properties of 80% (v/v) ethanolic extract of peanut sprout (PSE), which contains a high concentration of phenolic compounds such as resveratrol. For this, SD rats were orally administered PSE while a control group was incubated with saline. Laxative effects were examined in both groups of rats. Constipation induced by loperamide in SD rats was improved by administration of PSE. Constipated rats showed increased intestinal movement of $BaSO_4$ upon administration of PSE compared to the control, and the groups administered 100 or 1,000 mg PSE/kg bw were not significantly different in transit time of the indicator. However, colon length was not statistically different among the experimental groups, although it was longer in the group incubated with 1 g PSE/kg bw compared to other groups. Further, there was no significant difference in stool number among the experimental groups. Taken together, these findings show that PSE has a laxative effect in a rat model of loperamide-induced constipation.

Key Words: Peanut sprout, laxative effect, constipation

Introduction

Constipation is a common problem worldwide and contributes significantly to health care financial burden. The worldwide prevalence of functional constipation varies from 0.7% to 29.6% in children and from 2% to 35% in adults depending on the geographical region [1-3]. Constipation significantly affects quality of life as it can cause not only discomfort and restlessness but also abdominal distension, vomiting, gut obstruction, and perforation, and it is even associated with fatal pulmonary embolism [4]. As a majority of laxative drugs have side effects, it is worthwhile to search for food ingredients or herbs with laxative effects.

Peanut sprout has been reported to contain relatively high levels of phenolic compounds and resveratrol [5], and its ethanolic extract has antioxidant enzyme-inducing activity [6]. Specific polyphenols such as mangiferin and genkwanin 5-O-β-primeveroside have been reported to exert laxative effects [5,7,8]. Accordingly, this study was conducted to investigate whether or not peanut sprout extract enriched with polyphenols has a laxative effect.

Materials and Methods

Materials

Peanut (PoongAn cultivar) and its sprout were freeze-dried, powdered, and extracted with 40 volumes of 80% (v/v) ethanol solution as a solvent, along with agitation at 70℃ for 90 min. Extracts were then filtered, concentrated using a rotary evaporator, and freeze-dried prior to use as a test sample. Extraction yields were 2.7 and 23.4% for peanut and its sprout, respectively. Loperamide (Youngil Pharmaceutical Co.) was used to induce constipation at a dose of 2 mg/kg bw per day.

Animals

Male SD rats (8-weeks-old) weighing 200-250 g were purchased from Samtako Co. Ltd (Osan, Korea). Animals were tested for their general health condition and allowed to acclimate for 1 week before use. All animals were housed at a controlled room temperature and humidity (20-24.0℃, RH 50 ± 5%) under a 12/12 h light/dark cycle. Food pellets (Samtako Co. Ltd., Osan, Korea) prepared based on AIN-76 and tap water were provided

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ad libitum. All animal experiments were approved by the "Animal Ethic Committee of Wonkwang University (permission number WKU12-47)".

Experimental design

Fifty SD rats were divided into five groups with 10 animals per group: (1) normal, (2) negative control, (3) PS (100 mg/kg bw), (4) PS (300 mg/kg bw), (5) PS (1 g/kg bw). Constipation was induced by force-feeding with loperamide (2 mg/kg bw, Youngil Pharmaceutical Co., Seoul) twice a day for 7 days after orally administration of PSE for 4 weeks. Before induction of constipation, diet and water intakes were measured once a day.

Stool parameters

The frequency, weight, and water content of stools from each rat were measured at 1-week intervals before constipation induction as well as every day after constipation induction. The water content and dry weight of stools were measured once a day. The moisture content of stools was measured by drying stools at 70℃ for 24 h.

Induction of constipation

Rats were maintained on experimental diets for 4 weeks, after which constipation was induced through oral forced feeding of loperamide (Young-Il Pharmaceuticals Co. Ltd, 2 mg/kg bw) twice a day for 7 days.

Determination of total phenolics

Total phenolics were determined using Folin-Ciocalteu reagent [9]. Briefly, 30 mg of sample was extracted for 18 h with 50 mL of 75% ethanol at room temperature on an orbital shaker set at 200x rpm. The mixture was filtered through Whatman filter paper and used for total phenolics assay at a total volume of 50 mL. Four hundred microliters of extract was mixed with 50 μL of Folin-Ciocalteu reagent (previously diluted 5-fold with distilled water) and 100 μL of saturated sodium bicarbonate solution and then allowed to stand at room temperature for 1 h. The absorbance was measured at 750 nm. Results are expressed as gallic acid equivalents.

Determination of resveratrol and polydatin using LC-MS/MS

The concentrations of resveratrol and polydatin were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described, with some modification [10]. In brief, 100 mg of sample was dissolved in dimethylsulfoxide. This solution was diluted with methanol to obtain a final concentration of 10 mg/mL, after which 0.5 μL of the solution was analyzed in a Thermo TSQ Vantage Triple-stage quadrupole mass spectrometer (ThermoFishcher Scientific, San Jose, CA, USA) equipped with an electrospray ionization interface. Resveratrol and polydatin were separated on a Luna C18 column (2.0 mm i.d. × 100 mm, 2.1 μm particle size; Phenomenex). Gradient elution was carried out with acetonitrile containing 0.1% formic acid as mobile phase A and water containing 0.1% formic acid as mobile phase B. A non-linear gradient profile was applied as follows: 0-2 min, 20% A; 2-5 min, 20-60% A; and 5-8 min, 60% A. The column was re-equilibrated for 5 min in order to return to 20% A. The mobile phase was eluted using a Thermo Accela HPLC system (Thermo Fishcher Scientific, San Jose, CA, USA) at a flow rate of 0.2 mL/min. The mass spectrometer was operated in negative ionization mode. The operating conditions were as follows: capillary temperature, 350℃; vaporizer temperature, 300℃; and ionization voltage, 3,000 V. The collision gas was nitrogen at a nominal pressure of 1.5 mTorr, and the collision energy was set to 20 eV. Quantitation was performed by selected reaction monitoring (SRM) of the precursor ion as well as the related product ion for resveratrol and polydatin using an external standard to establish peak area ratios. The mass transitions used for quantitation of resveratrol and polydatin were *m/z* 227→143 and 389→227, respectively. The retention times for resveratrol and polydatin were 6.1 and 4.6 min, respectively.

Statistical analysis

Values are presented as the mean ± SEM (standard error of the mean). Measurement data were analyzed by one-way ANOVA, followed by Duncan's multiple range post hoc test if overall differences were significant ($P < 0.05$). All statistical analyses were performed using SPSS 12.0 statistical software (SPSS Inc., Chicago, IL), and a difference was considered significant at $P < 0.05$.

Results

Total phenolic and resveratrol contents in peanut sprout extract

The dried peanut sprout was powdered and subjected to extraction with 80% (v/v) aqueous ethanol solvent, with the yield of 20.3 ± 3.1 % on a dry basis. The content of total phenolics present in peanut sprout extract was 390 ± 20 mg/g (Table 1). This value is much higher than the total phenolic content of peanut (1.52 mg/g), which is consistent with the other report [5].

Table 1. Total phenols and resveratrol contents in 80% ethanolic extracts of peanut and its sprout

	Peanut	Peanut sprout
Total phenols (%)	21.3 ± 1.6	39.0 ± 1.8
Polydatin (μg/g)	1.18 ± 0.01	2.23 ± 0.01
Resveratrol (μg/g)	0.037 ± 0.00	0.063 ± 0.01

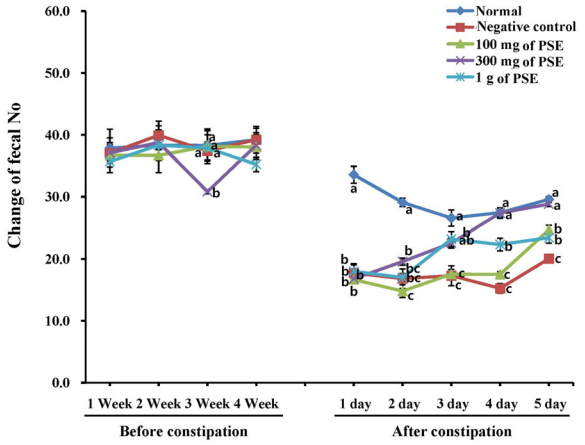

Fig. 1. Effects of peanut sprout extracts on changes in fecal number in loperamide-induced constipation model. Data are the means ± SD (n = 9). Bars with different letters from the control are significantly different (P< 0.05).

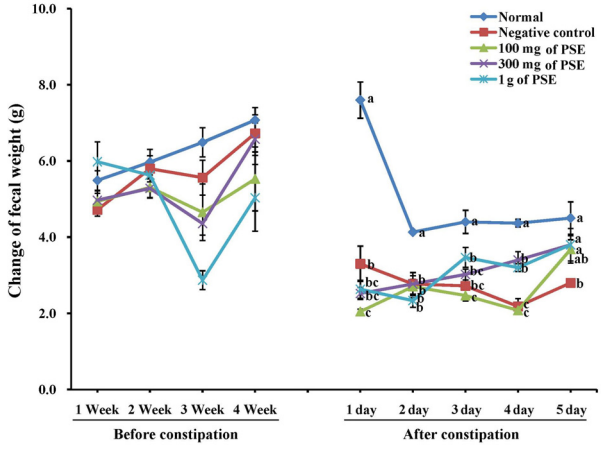

Fig. 2. Effects of peanut sprout extracts on changes in fecal weight in loperamide-induced constipation model. Data are the means ± SD (n = 9). Bars with different letters from the control are significantly different (P< 0.05).

Body weight change and diet and water intakes

Body weight changes among experimental groups were not significantly different during the 4-week experimental period. There were no significant differences in diet or water intake among the experimental groups both before and after constipation induction.

Effect of PSE on stool parameters

To examine the laxative effects of PSE on frequency and wet weight of stools in normal male rats, PSE (100, 300, and 1,000 mg/kg) was orally administered once daily for 4 weeks. The frequency and wet weight of stools were measured every week before constipation induction and daily after constipation induction. Stool frequency was not significantly different among the experimental groups before constipation induction. However, stool frequency decreased after constipation induction in all groups.

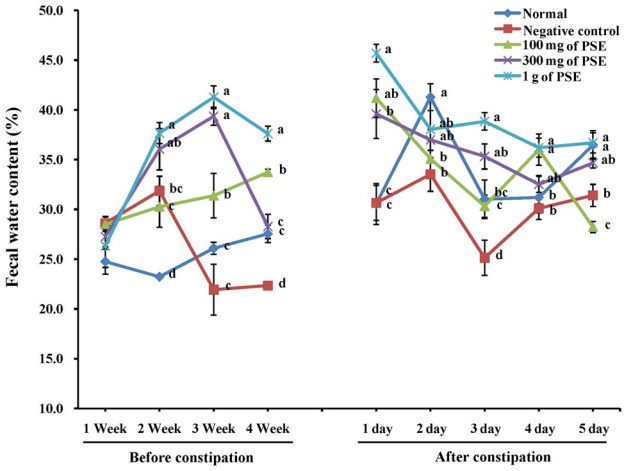

Fig. 3. Effects of peanut sprout extracts on changes in fecal water content in loperamide-induced constipation model. Data are the means ± SD (n = 9). Bars with different letters from the control are significantly different (P< 0.05).

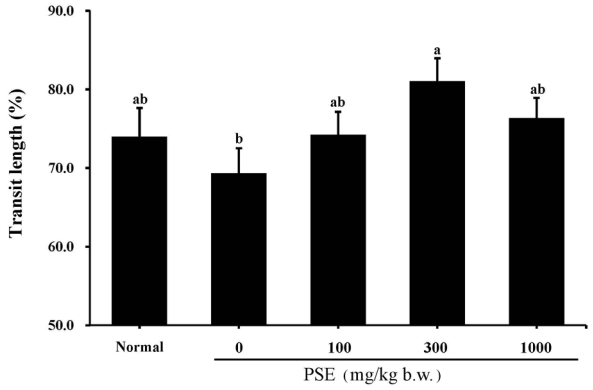

Fig. 4. Effects of peanut sprout extracts on changes in intestinal length in loperamide-induced constipation model. Data are the means ± SD (n = 9).

Whereas the control group showed the lowest fecal number, rats administered 300 and 1,000 mg PSE/kg bw showed gradual increases in stool frequency starting from the first day of constipation induction (Fig. 1).

Meanwhile, fecal weight was not significantly different among the experimental groups before constipation induction. However, fecal weight significantly increased in groups fed a diet containing 300 or 1,000 mg PSE/kg bw (Fig. 2). The fecal water contents of these groups were significantly higher than that of the control (Fig. 3). However, fecal water content in the colon was not significantly different among the experimental groups.

Effect of PSE on small intestinal transit rate in loperamide-induced constipated mice

Although there was no significant difference in small intestinal length among the experimental groups, the constipation-induced group fed a diet of 300 mg PSE/kg bw showed faster intestinal movement of $BaSO_4$ than the negative control, whereas groups

fed a diet of 100 or 1,000 mg PSE/kg diet showed no difference in transit time of the indicator (Fig. 4). However, colon length was not statistically different among the experimental groups although it tended to be longer in the 1 g PSE diet group than the others. Further, there was no significant difference in stool number among the experimental groups.

Discussion

Constipation, which is defined as the infrequent and difficult passage of stools in the absence of any physiological abnormality [11], is a common condition affecting the gastrointestinal (GI) tract, with higher prevalence in women, the elderly, and those with lower income [12,13], and is associated with reduced quality of life [14]. Treatment of constipation usually consists of a four-step approach involving education (including information about the prevalence and pathophysiology of constipation and the necessity of long-term treatment), disimpaction, prevention of re-accumulation of feces, and behavioral therapy. Long-term follow-up also seems to be critically important for treatment success [15,16]. Drugs containing magnesium oxide or sennoside, the main constituent of senna, are typically administered for treatment of constipation due to their powerful purgative/laxative activities, but these drugs also induce various side effects such as severe diarrhea and abdominal cramping.

Dietary interventions are also considered as a treatment option for the management of constipation. The most widely studied ingredients for preventing and/or treating constipation include probiotics and fiber [15]. The mechanisms of action through which probiotics prevent or treat constipation have not been fully elucidated. One hypothesis is that there is an imbalance in the normal gut microflora in constipated patients that could be improved by ingestion of probiotics. Another possible mechanism is decreased colonic transit time via reduction of colonic pH induced by probiotics [17]. The suppressive role of fiber against constipation is most likely related to its ability to induce osmotic and mechanical stimulation of colonic motility [18]. In particular, insoluble dietary fiber has been shown to increase fecal bulk, increase water content of stool, accelerate the number of bowel movements, and promote softer consistency, all of which help alleviate constipation [19].

Peanut sprout has attracted much attention due to the presence of resveratrol, which has a variety of bioactive functions. In our study, PSE was found to contain 2.23 μg/g piceid, 0.06 μg/g resveratrol, and 39% total phenolics. Our results show that PSE increases intestinal transit time and fecal frequency without body weight changes and any deleterious effects.

Although PSE showed a laxative effect in the loperamide-induced constipation model, its laxative mechanism is not clear. It has been reported that some phytochemicals could play a key role in laxative activity via acetylcholine receptors [20]. There is a possibility that phytochemicals can stimulate bowel movements by inhibiting carbohydrate-digesting enzymes, thereby increasing fecal volume [21,22]. As the administration of PSE increased fecal frequency and weight without significantly affecting fecal water content, the laxative action of PSE is most likely associated with inhibition of digestive enzymes. However, it is also possible that PSE exerts its laxative action through interactions with acetylcholine receptors. In fact, the mouse model of loperamide-induced constipation corresponds to morphine-induced constipation in human patients since both loperamide and morphine are opioid-receptor agonists [20]. The plant constituent genkwanin-5-O-beta-primeveroside isolated from Agarwood has been reported to increase the intestinal contractions of the ileum isolated from guinea pigs via action on acetylcholine receptors.

Taken together, PSE showed a protective effect in a loperamide-induced animal model without apparent side effects. Therefore, PSE deserves further study as a constipation therapeutic agent.

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